FREQUENCY OF NOTCH MUTATIONS INDUCED IN NORMAL, DUPLICATED AND INVERTED X-CHROMOSOMES OF DROSOPHILA MELANOGASTER

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AMONG spontaneous and X-ray-induced effects at specific loci in *Drosophila melanogaster,* Notch mutations are particularly frequent. The first Notch (*N,* locus 1–3.0) was found by DEXTER (1914), and Notch mutants were early studied and described by MORGAN (1919) and MOHR (1923 and 1932). In more recent years GOTTSCHEWSKI (1937), DEMEREC (1939, 1940 and 1941), and DEMEREC and FANO (1941) have analyzed many different Notch mutants, including a cytological examination of the salivary chromosomes (see descriptions in BRIDGES and BREHME 1944). From these studies much is known about the Notch mutation. Notch is sex-linked, lethal in the male and homozygous female. In the heterozygous condition females characteristically exhibit excisions or notching of the posterior margin of the wings, as well as other less obvious differences from the wild-type condition. All Notches behave genetically as though they were deficiencies. Not only are they male-lethal but they also invariably " uncover " two recessive genes, split (*spl,* 3.0) and facet (*fa,* 3.0), and frequently other nearby genes as well, such as prune (*pn,* 0.8), white (*w,* 1.5), roughest (*rst,* 1.7), diminutive (*dm,* 4.6), and echinus (*ec,* 5.5).

Split and facet alleles affect the size and surface structure of the eye; in addition *spl* typically produces doubled bristles (hence its name), whereas *fa* shows some degree of wing nicking, particularly the allele fa^n. Both *spl* and *fa* are male-viable, but the combination *spl/fa* is wild-type in appearance. Thus, the two genes do not act like alleles. Still, no crossing over occurs between them, and the exact relationships between *N, spl* and *fa* remain unclear.

DEMEREC, SUTTON and HOOVER have analyzed the salivary chromosomes of a large number of Notch mutants, many of which are described in BRIDGES and BREHME (1944). Their analyses showed that many induced as well as spontaneous Notch mutants are associated with a cytological detectable deficiency. With few exceptions the deficiency includes salivary chromosome band 3C7 of BRIDGES' (1938) map. However, in at least three cases, $N^{264\text{-}79}$, $N^{264\text{-}86}$ and $N^{264\text{-}90}$, band 3C7 appears not to be included in the deficiency.

Although many of the same Notches listed in BRIDGES and BREHME have been reported by DEMEREC (1941) and DEMEREC and FANO (1941), a summary of the entire group will be revealing. In table 1 are presented the Notch mutants from BRIDGES and BREHME about which cytological or crossing over data are given. The tabulation shows the percentages of different categories of Notch mutants, both spontaneous and X-ray induced. It may be seen that

spontaneous Notches differ from X-ray-induced ones in two important respects: a) when deficiencies are detectable, those arising spontaneously tend to be shorter than those resulting from irradiation, and b) spontaneous Notches arising in association with translocations or inversions are very rare. Among the Notches not associated with gross rearrangement, a considerable number show no cytologically detectable deficiency. Even when a deficiency is evident, frequently only one band, 3C7, is missing. Indeed, among the X-ray Notches about 30% are deficient for one band or less, even when those non-deficient mutants associated with rearrangements are not considered.

About one-third of all induced Notches are associated with gross rearrangements; however, a few of these (3 out of 36) show a detectable deficiency. Most of the rearrangements, to be sure, bring band 3C7 near heterochromatin, and the resulting Notch mutants may be ascribed to position effect rather than to gene loss. SCHULTZ (cited in BRIDGES and BREHME 1944) demonstrated that the addition of an extra Y chromosome resulted in the production of

TABLE 1

Summary of Notch mutants recorded in BRIDGES and BREHME (1944) about which cytological or crossing over data are given.

Total No.	White-Notch	Notch-dimin.	Cytol. normal	1 band deficient	2–19 bands deficient	20–50 bands deficient	Rearrangements (non-deficient)
				Spontaneous			
29	13.8%	20.7%	23.5%*	23.5%*	53.0%*	0	0
				X-ray-induced			
95	15.8%	22.1%	16.9%	13.7%	17.9%	16.9%	34.7%**

* Only 17 of the 29 spontaneous Notches were studied cytologically.
** Five additional mutants associated with rearrangements (inversions or translocations) have been excluded because some viable non-Notch males were produced.

viable, though sterile, males in the case of three different Notches, each associated with a translocation which brought band 3C7 close to heterochromatin ($N^{264\text{-}6}$, $N^{264\text{-}9}$ and $N^{264\text{-}10}$).

In none of the deficient Notches does the loss exceed 50 bands (DEMEREC 1941). Therefore, deficiencies of greater extent probably act as dominant lethals, and do not survive even in heterozygous condition. POULSON (1945) studied the embryological development of various Notch males, some with no detectable deficiency and some with maximum loss, and he concluded that the lethal action was similar in both types of Notches. However, no correlation exists between the extent of the deficiency and the degree of expression of the Notch phenotype.

The question exists: is a "no-band" Notch the result of a true lethal gene mutation, or, like a detectably deficient Notch, is a cytologically normal Notch associated with a real loss of genetic material, the loss being too slight to be observable in the salivary chromosomes? Since all Notches behave genetically like deficiencies, the latter interpretation is favored. On the other hand DEMEREC

(1941) put forth the view that in cytologically normal Notches the Notch "gene" has been inactivated (lost its specificity) but that no chromatic material is actually missing. The fact that position-effect Notches exist suggests that genes can be inactivated without loss. Probably the best experimental way to distinguish between these two interpretations would be to attempt to produce reverse mutation of various cytologically normal Notch mutants which are not associated with gross rearrangements. A reversible Notch mutant could not easily be regarded as caused by a genetic loss (cf. MULLER 1930). However, even if the mutational nature of some Notch mutants were demonstrated, any lethal mutation, inactivation, or loss of the N^+ locus should give rise to the typical Notch phenotype.

How then are *fa* and *spl* related to *N*? If *spl* and *fa* are both allelic to *N*, then *spl* and *fa* must be regarded as non-lethal mutational changes of the N^+ locus, and cannot be losses. However, at least one lethal *fa* allele has been reported by MULLER and ALTENBERG (1921). Actually, *spl* and *fa* appear not to be alleles, but they possibly could be pseudo-alleles. If so, then the wild-type condition would be spl^+ fa^+ (cf. BAUER 1943). Now, both viable and lethal *spl* and *fa* alleles may be visualized, but lethal *N* mutants would result only from the loss, inactivation, or simultaneous mutation of *both* loci. In this view a mutational Notch would be very rare indeed, except under some hypothesis of chain mutation, which itself appears most unlikely (GOTTSCHEWSKI 1937). In summary, it is difficult to believe that even cytologically normal Notch lethals can result from true gene mutation, and the same may hold for any lethal at a locus where viable alleles are known. On the other hand the occurrence of viable mutants which result from actual loss rather than from mutation has been demonstrated by MULLER (1935) and others. Thus, the entire series of X-ray induced *N* changes could be visualized on the basis of genetic loss of varying extent.

Two duplications are known which possess in common the ability to suppress the Notch phenotype when compounded with Notch mutants. These are Abruptex (*Ax*) and Confluens (*Co*), described in BRIDGES and BREHME (1944). *Ax* is a one-band duplication, having two bands in place of the normally single 3C7 band (MORGAN, SCHULTZ and CURRY 1941). *Ax* itself is expressed mainly by a shortening of one or more longitudinal wing veins. In combination with *N* the shortened wing veins are commonly observed, but the wings are usually not notched. A small percentage of *Ax/N* individuals do possess Notch wings, however. *Co* is a longer duplication than *Ax*, involving bands 3C5 through 3D6 (SCHULTZ 1941). *Co* is expressed by thickened, but not shortened, wing veins; but *Co/N* is nearly wild-type in appearance. It may be noted that *N* itself shows slightly thickened wing veins. MORGAN, SCHULTZ and CURRY (1941) found that crossing over can occur between a deficiency Notch and *Co*, producing a duplication that no longer has two N^+ loci but is otherwise identical with the original *Co* duplication. The *Co* phenotype is then no longer expressed. Therefore, they concluded that the duplication of the Notch locus produces the *Co* phenotype. Since the *Ax* phenotype is different

from *Co*, the Abruptex effect must result from some sort of position effect on band 3C7 rather than from a true duplication of the Notch locus. The extra band in *Ax* may not be 3C7; if it is, then the Notch locus may not be in 3C7.

Despite the large number of Notch mutants produced by DEMEREC, remarkably few quantitative data on the X-ray induction of Notches appear in the literature. Some indication of the Notch mutation frequency following various doses may be derived from the studies of DEMEREC (1934), MULLER (1940), and LEWIS (1945). However, SITKO (1938) demonstrated that the induced Notch mutation rate can be appreciably increased if band 3C7 is brought close to heterochromatin, as occurs in the white-mottled-4 (w^{m4}) inversion.

The present study is an attempt to analyze the X-ray production of Notch mutations by comparing their incidence in structurally normal chromosomes with that in abnormal chromosomes. A given dose of X-rays (5000 r) was applied to Canton-S wild-type males, and the number of induced Notch mutants was determined. A similar dose was then applied to Abruptex males in order to determine what effect the presence of the extra band in *Ax* has on the production of Notch mutants. Finally, the same dose was applied to w^{m4} and roughest-3 (rst^3, an inversion rather similar to w^{m4}) males in order to observe what effect bringing band 3C7 close to heterochromatin by means of inversions has on the production of Notch mutations. In each case any simultaneous occurrence of a white effect accompanying the Notch mutation was recorded. Moreover, a considerable amount of data was obtained regarding the induction of male-viable split mutations.

The results of these experiments show that the Notch mutation rate in wild-type and *Ax* males is similar. Likewise, the incidence of Notches is the same in w^{m4} and rst^3; but in the latter two stocks the frequency of Notch mutants is more than three times higher than that observed in the non-inversion stocks. Thus, *Ax* appears not to contain a duplication of the Notch locus, and nearness of N^+ to heterochromatin markedly increases the frequency of induced Notch mutations. However, no male-viable *spl* mutations were observed.

MATERIALS AND METHODS

Four stocks of *Drosophila melanogaster* were used to provide males for irradiation: 1. Canton-S wild-type, 2. yellow-2, apricot, Abruptex ($y^2\ w^a\ Ax$), 3. white-mottled-4 (w^{m4}), and 4. yellow, roughest-3, bobbed, carnation ($y\ rst^3\ bb\ car$). The latter two stocks contain inversions bringing the Notch locus close to the proximal heterochromatin (see fig. 1). Males from each stock were placed in size 000 ventilated gelatin capsules for irradiation. A dose of 5000 r was given in all cases. Two different X-ray machines were used in the course of the work. The first, used for most of the Canton-S + and for about half of the w^{m4} exposures, was an oil-cooled G.E. Maximar deep therapy machine, operated at 200 KV and 25 ma at 20 cm target distance, unfiltered. This setting produced an X-ray intensity of 650 r/min as measured by a Victoreen dosimeter, corrected for the barometric pressure. This correction, usually negligible, is significant at the altitude of Salt Lake City, Utah: 4366 feet. The

second machine, used for all other exposures, was a Westinghouse Quadracondex water-cooled deep therapy machine operated at 250 KV and 15 ma at 35 cm target distance, unfiltered. This setting produced an intensity of 425 r/min. The mutational similarity of 5000 r doses delivered from each machine was checked by extensive lethal tests. Canton-S + males were exposed to 5000 r from the first machine and mated with Muller-5 females. In 3,970 F_2 cultures checked, 519 recessive sex-linked lethals were found, an incidence of 13.1%. A presumably equivalent dose from the second machine given to *rst*^{3}

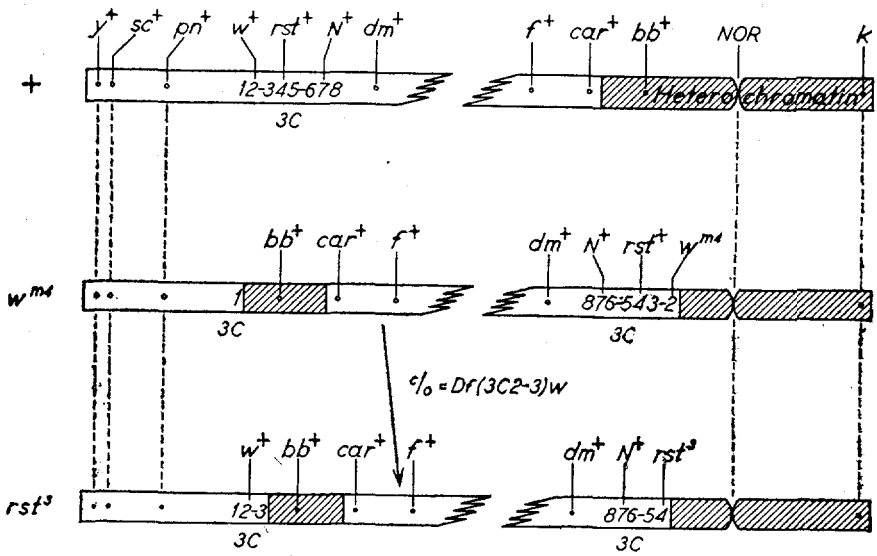

FIGURE 1.—Diagrammatic representation of wild-type, white-mottled-4 (w^{m4}), and roughest-3 (rst^3) mitotic X-chromosomes, showing the relationships to heterochromatin of various gene loci: yellow (*y*), scute (*sc*), prune (*pn*), white (*w*), roughest (*rst*), Notch (*N*), diminutive (*dm*), forked (*f*), carnation (*car*), and bobbed (*bb*) and also of the nucleolus organizing region (NOR) and the kinetochore (k). The salivary band locations of *w*, *rst*, and *N* are indicated. The euchromatic and heterochromatic break points of the inverted X-chromosomes follow BRIDGES and BREHME (1944) and KAUFMANN (1942 and 1944).

males produced 125 sex-linked lethals in 991 F_2 cultures checked, an incidence of 12.6%. Thus, no significant difference existed in the output of the two machines. Approximately 13.5% sex-linked lethals would be expected from 5000 r, according to MULLER (1950). Possibly the dose actually delivered by the two X-ray machines was somewhat less than 5000 r.

The irradiated males were mated to females whose genotype permitted the detection of white as well as Notch mutations in the F_1 females. In addition, females mated with Canton-S + and *Ax* males possessed inversions in order to prevent crossing over between the irradiated paternal and the maternal X-chromosomes. Since the w^{m4} and rst^3 males themselves had inversions, they were mated with females having normal X-chromosomes, but which carried the recessive gene *spl* in order to facilitate the detection of Notch mutants.

The female F_1 offspring were examined individually for Notch and white mutations under a binocular dissecting microscope at a magnification of 15 or 18 diameters. When a female showing mutant eye color or notching of the wings was found, she was placed in a vial with brothers and allowed to breed. Verification of the mutation was possible when a stock could be developed from the original female for breeding tests. In particular, most of the Notch mutants were proven by mating with special N^+ duplication males. These males possessed a short segment of X-chromosome containing the w^+ and N^+ loci inserted in an autosome. True Notch mutations were covered by this duplication; mimicking mutations were not covered.

However, a number of presumptive Notch and white mutants, having good phenotype, for one reason or another failed to produce offspring for testing. These non-breeding mutants were taken into account in calculating the mutation rates recorded in the next section. A proportion of the sterile mutants equal to the proportion of the fertile mutants which were proven by breeding test to be true white or Notch mutations was added to the proven mutants in order to obtain the overall mutation rates.

Further determined in all cases was the fraction of all Notch mutations which simultaneously affected the white locus.

RESULTS

In table 2 are recorded the complete summarized results of the production of Notch, white and split mutations following irradiation with 5000 r of Canton-S +, *Ax*, w^{m4} and rst^3 males. It may be noted that between 22,000 and 27,000 F_1 females were examined in each series. In table 3 the same data are presented, expressing the frequency of Notch, white and split mutations expected if exactly 25,000 F_1 females had been examined and taking into account the sterile mutants of each kind.

The results as seen in table 3 are clear. The total number of Notch mutations produced in Canton-S + was quite similar to that in *Ax*, being similar moreover to the total number of white mutations in each case. Furthermore, the Notch mutation frequency was the same in w^{m4} as in rst^3, but considerably exceeded that found in either Canton-S + or in *Ax*. It may be further noted that in w^{m4} white-Notches constituted a large fraction of all Notch effects, whereas in rst^3 no white-Notch mutants whatsoever were found because w^+ and N^+ are distantly separated in rst^3, unlike the relationships in w^{m4} (see fig. 1). In w^{m4} those Notches which were not simultaneously white occurred with a frequency slightly, but not significantly, higher than in Canton-S +. However, it must be remembered that detection of *N* mutants was facilitated in w^{m4} (and in rst^3) by the presence of *spl* in the parental females.

It seems fair to combine the Canton-S + and *Ax* data with regard to Notch mutation frequency; likewise the w^{m4} and rst^3 data. Following exposure to 5000 r, then, Notch mutations were produced in the non-inverted chromosomes with a frequency of nearly one per 1000 (44 in 46,570) irradiated X-chromosomes; similarly, white mutations occurred with a frequency of about one per

TABLE 2

Male-viable, male-lethal, and sterile mutants detected in F_1 females following irradiation of Canton-S+, Ax, w^{m4}, and rst^3 males with 5000r.

Mutant	Breeding results	Males irradiated			
		+	*Ax*	w^{m4}	rst^3
White	Viable	6	12	23	10
	Lethal	5	3	14	23
	Sterile	9	7	8	10
White-Notch	Viable	0	0	0	0
	Lethal	3	2	44	0
	Sterile	1	0	16	0
Non-white Notch	Viable	0	0	0	0
	Lethal	12	13	21	58
	Sterile	8	5	4	31
Split	Viable	Not detectable		0	0
No. F_1 ♀♀ examined		24,261	22,309	26,164	26,723

1000 (48 in 46,570). However, in the inversion stocks, Notch mutations occurred with a frequency of one per 300 (174 in 52,887) irradiated X-chromosomes following exposure to 5000 r.

In the F_1 females examined following exposure of w^{m4} and rst^3 males, split as well as Notch mutants could be detected because of the presence of *spl* in the parental females. Altogether, 52,887 F_1 females heterozygous for *spl* were examined but not one male-viable *spl* mutant was observed. Every apparent split effect was, on breeding test, a male-lethal Notch mutant. Of the 174 F_1 females which did have split eyes, 123 were fertile and were proved to be Notch mutants. Among the 51 sterile mutants, it is conceivable that one (or more) might have proved to be a male-viable split mutant, rather than a Notch, had it been fertile. However, the great majority of these sterile mutants showed the typical wing nicking of Notch. Since the fertile ones, whether or not they had obviously notched wings when first observed, invariably bred as

TABLE 3

Expected frequency of all white (male-viable and lethal), white-Notch, non-white Notch, all Notch, and male-viable split mutants following irradiation of Canton-S +, Ax, w^{m4}, and rst^3 males with 5000r, assuming the examination of 25,000 F_1 females and taking into account sterile mutants of each kind.

	Males irradiated	All white mutants	White-Notch mutants	Non-white Notch mutants	All Notch mutants	Viable split mutants
Non-inverted X-chromosomes	+	24.7	4.1	20.6	24.7	....
	Ax	27.0	2.2	20.2	22.4	....
Average		25.8	3.2	20.4	23.6	....
Inverted X-chromosomes	w^{m4}	100.2	57.3	23.9	81.2	0
	rst^3	40.2	0.0	83.3	83.3	0
Average		....	....	....	82.2	0

male-lethal Notch mutants, it seems unlikely that X-rays are effective in inducing male-viable *spl* mutations.

DISCUSSION

Four experimental determinations were made in the present investigation of the X-ray induction of Notch mutations: 1) the frequency of Notch mutations produced in wild-type X-chromosomes by an irradiation of 5000 r, 2) the Notch frequency produced in Abruptex X-chromosomes by the same dose, 3) the Notch frequency in inverted X-chromosomes in which salivary chromosome band 3C7, which presumably contains the N^+ locus, lies close to proximal heterochromatin, and 4) the frequency of male-viable split mutations.

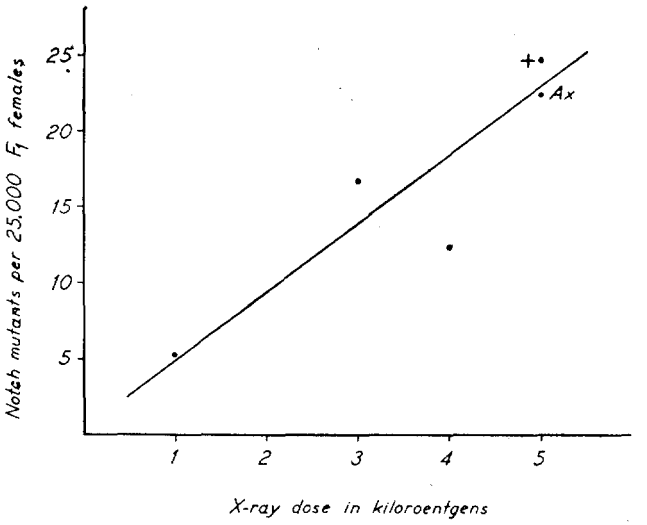

FIGURE 2.—Relationship between X-ray dosage and the production of Notch mutations. The frequency of Notches is expressed as the number expected per 25,000 F_1 females examined. Data at 1000r and 4000r from MULLER (1940), at 3000r from LEWIS (1945), and at 5000r from the present study.

In the irradiation of Canton-S +, Notch mutants occurred with a frequency of approximately one per 1000 irradiated X-chromosomes; one-sixth of the Notch mutations were accompanied by an effect at the white locus. Before these results may be accepted as a basis for comparison with the other irradiations, it is desirable to determine if independent investigations on the frequency of X-ray-induced Notches are in agreement with these results. MULLER (1940) and LEWIS (1945) reported values for the induction of Notches following exposure to 1000 r, 3000 r, and 4000 r. It is not clear whether they included in their values sterile as well as fertile mutants; in any event the detection of Notches was not the primary focus of attention in their experiments. Thus, precise agreement as to the frequency of Notch mutations cannot be expected. However, as can be seen in figure 2, their data combined with those from the present experiment indicate fairly well that Notch mutants are produced with

a frequency proportional to dose. MULLER (1940) believed that mutants such as Notch, even though many are deficiencies, result from the occurrence of single X-ray hits, accompanied by a spread of effect of greater or lesser extent. The combined results depicted in figure 2 support that view for Notch mutations. DEMEREC (1934) also reported data on Notch mutation frequency following exposure to 2500 r. However, not only his values for *N* mutants, but also those for mutants at various other loci, were consistently low in comparison with other studies of mutation frequency. Therefore, his data have not been included in figure 2.

The fraction of Notch mutants which are simultaneously white was not reported by MULLER or LEWIS. However, the Notches listed in BRIDGES and BREHME (1944) may serve for comparison with the present study (see table 1). It must be recognized that two types of accompanying white effects may be present; 1) white eyes, and 2) white-mottled eyes. Only the former, Notches which have pure white eyes, are concerned in this comparison. In the Canton-S + exposures, four of the 24 Notches had white eyes, or 16.7%. Of the 95 cytologically studied induced Notches listed in BRIDGES and BREHME, 15 had white eyes, or 15.8%.

It may be accepted that the results of the Canton-S + irradiation are not significantly different from those of other investigators and may be safely used for comparison with the irradiations of the stocks possessing structurally abnormal X-chromosomes.

The frequency of Notch mutants resulting from the irradiation of *Ax* with 5000 r was quite similar to that produced from Canton-S +, as seen in table 3. However, only two of the 20 Notch mutants found in the *Ax* experiment were white-Notches, while four of the 24 from the Canton-S + irradiation were white-Notch. The numbers are too small to ascribe a significance to this difference. It should be noted that in the combined results of the Canton-S + and *Ax* irradiations six of the 44 induced Notch mutations, or 13.6%, were white-Notch, which is in good agreement with the data from BRIDGES and BREHME (1944).

The fact that the frequency of Notch mutations induced in *Ax* is no different from that in wild-type implies that *Ax* contains no true duplication of the N^+ locus. Thus, the inference of MORGAN, SCHULTZ and CURRY (1941) that *Ax* results from a position effect rather than from a duplication of the N^+ locus is supported by these mutation studies. Nonetheless, *Ax* is effective in suppressing the Notch phenotype in the combination: *Ax*/*N*.

If the extra band in *Ax* suppressed the Notch phenotype as well when *N* is induced in the *Ax* chromosome as it does when *N* is present in the opposite chromosome, then fewer Notches would be expected following irradiation of *Ax* than of wild-type because all no-band and one-band Notches would not be detectable in *Ax*. However, position-effect Notches (showing no deficiency) might well be equally frequent in *Ax* as in + because the position effect could inactivate two adjacent 3C7 bands as well as one. From a consideration of the 95 induced Notches listed in table 1 it may be concluded that the following

three classes of Notch mutants are about equally frequent: a) Notches which are not associated with gross rearrangement and which have a detectable loss of one band or less (29), b) Notches having a detectable loss of two bands or more (33), and c) non-deficient Notches associated with gross rearrangements, giving rise to a position effect (33). Thus, about 30% fewer Notches would be expected from the irradiation of *Ax* than from +.

However, the extra band in *Ax* does not produce a reduction in the detected number of induced Notches; thus, no-band and one-band Notches induced in the *Ax* X-chromosome are not suppressed. Therefore, the usual suppression of the Notch phenotype by *Ax* must result from a position effect on the N^+ locus such that even in a single dose it becomes effective in giving rise to wild-type wings in a female just as a single N^+ locus normally does in a male. The Notch phenotype ordinarily observed in N/N^+ females cannot be a consequence of the absence of one N^+ locus, but rather is the usual expression of a single N^+ locus in a female. The single N^+ locus in the *Ax* chromosome becomes as effective as two N^+ loci in producing normal wings in *Ax/N* females. However, loss of the N^+ locus from *Ax* must give rise to a Notch phenotype just as it would in a wild-type chromosome because the extra band in *Ax* does not substitute for N^+; that is, the extra band does not contain a duplicated N^+ locus.

Although the *Ax* duplication surely does not contain two N^+ loci, the nature of the extra band present is uncertain. The appearance of the *Ax* salivary chromosome suggests that two 3C7 bands are present (MORGAN, SCHULTZ and CURRY 1941). If so, then the N^+ locus is not located in 3C7. However, the extra band may be some entirely unrelated band which has been inserted next to 3C7. If so, then the N^+ locus may well be contained in band 3C7. No information from the present study serves to distinguish between these two possibilities.

The phenotype associated with a true duplication of N^+ is a thickening of wing veins as seen in Confluens. Moreover, duplications of the N^+ locus which by insertional translocation are present in autosomes, rather than being adjacent to the normal N^+ locus as in Confluens, show a thickened wing vein phenotype similar to that of *Co*. Three such insertional N^+ duplications were used in proving presumptive Notch mutants produced in the present study. All three show a Confluens-like phenotype when present in addition to a normal X-chromosome. However, when present with a Notch mutation in a male, not only may the male survive, but it ordinarily shows neither wing nicking nor Confluens-like wing veins.

In non-inverted X-chromosomes, effects at the Notch locus occurred following 5000 r irradiations with a frequency of approximately one per 1000. However, bringing the N^+ locus close to proximal heterochromatin by means of the w^{m4} and rst^3 inversions increased the frequency of induced Notch mutations more than three-fold. Assuming that the N^+ locus is in band 3C7, then in w^{m4} the N^+ locus is separated from heterochromatin by five bands; in rst^3 by only three bands (see fig. 1). In each of these inversions a position effect is evident

on the expression of genes immediately next to the heterochromatin; however, no change in N^+ is apparent in either inversion. Still, it could be imagined that a position effect on mutability of the N^+ locus is present, even though no effect on phenotypic expression is evident. On the other hand, the proximity of N^+ to heterochromatin may serve to facilitate the production of heterozygous viable deficiencies of the N^+ locus. In normal X-chromosomes, Notch deficiencies exceeding 50 bands do not survive even as heterozygotes (DEMEREC 1941). Should the bulk of deleted material be heterochromatin, however, then deficiencies of much greater actual extent could survive to be detected.

The results of the w^{m4} irradiation serve to distinguish between these two possible interpretations. In w^{m4} two classes of Notch mutations can be observed: a) Notches not simultaneously accompanied by a white effect, and b) white-Notches. If a position effect on mutability of N^+ were present in w^{m4}, then both classes of Notch mutations should be increased in comparison with those produced by irradiating wild-type X-chromosomes. However, the actual data show that nearly all of the increased number of Notch mutations in w^{m4} were white-Notches. Notches which were not white, thus not including any heterochromatic deficiency, occurred with a frequency of about one per 1000, similar to the frequency of Notches induced in Canton-S +. Therefore, the proximity of N^+ to heterochromatin, as in w^{m4} and in rst^3, increases the likelihood that a Notch deficiency will survive as a heterozygote, and does not reflect any position effect on mutability. Such an increase in survival must result from the fact that a greater number of Notch deficiencies which lack fewer than 50 euchromatic bands are produced in w^{m4} and rst^3 than in +. Proximity of N^+ to heterochromatin would not of itself produce more such deficiencies if there were some physical limitations so that X-ray induced deficiencies could never exceed a distance equivalent to 50 bands. The greater number of Notch mutants induced in the inversion stocks clearly implies that numerous deficiencies of N^+ have a total extent exceeding a distance equal to 50 bands, but a sufficient proportion of that total extent is heterochromatin, thus restricting the euchromatic loss to less than 50 bands.

Such a view of the effect of the proximity to heterochromatin should apply to any euchromatic locus which by inversion has been brought close to heterochromatin. MULLER (1940) reported the effect of irradiating the scute-8 (sc^8) inversion. In sc^8 the y^+ locus is adjacent to a large segment of heterochromatin. Muller recorded 73 yellow mutants among 22,330 F_1 females following irradiation of sc^8 males with 4000 r, which is at least three times as many as would be expected from a similar irradiation of non-inverted X-chromosomes. SIDOROV (1936) performed a similar study on sc^8 and noted that of 73 fertile yellow mutants, only 11 were male-viable. Of these 11, moreover, nine were yellow-achaete mutants; that is, not effects at the y^+ locus alone but involving the adjacent ac^+ locus as well. Such a $y\,ac$ mutant is likely a male-viable deficiency, similar to that produced by MULLER (1935), even though SIDOROV considered the $y\,ac$ mutants to be true double mutations. Thus, at the yellow locus, like the Notch, proximity to heterochromatin facilitates deletion because

of the " inertness " of heterochromatin. In addition, BELGOVSKY and MULLER (1938) noted numerous changes at the forked (*f*) locus following irradiation of the B^{M2} inversion.

The fact that detectable deletions are more numerous following irradiation of the inversion stocks further implies that a considerable number of deletions too long to survive as heterozygotes are produced following irradiation of normal chromosomes, even though they are ordinarily undetectable. Interestingly enough, one of the Notch mutants produced from Canton-S + in this experiment was just such an excessively long deficiency which was detected because the deleted material was simultaneously inserted in the third chromosome. Examination of the salivary chromosomes showed that the X-chromosome is deficient from approximately band 1E2 to 3C8. This Notch deficiency (N^{50k11}), extending some 70 bands, does not survive unless accompanied by the insertion, which itself is slightly shorter than the deficiency. DEMEREC (1940) described some insertional translocations of the white-Notch region which were even longer. Thus, the production of dominant lethal deficiencies incapable of surviving in heterozygotes is a normally occurring, and probably not infrequent, consequence of irradiation.

DEMEREC and FANO (1941) considered that Notch deficiencies less than about 15 bands (counting doublets as one band) in length resulted from one-hit effects; longer ones from two-hit effects. If this is true, then Notches produced in w^{m4} and rst^3 should show a non-linear relationship with dose since the great majority of them must be deficient for more than 15 bands. On the other hand, MULLER (1940) found that yellow mutants produced from sc^8 (an analogous situation) followed a linear rather than an exponential relationship with dose. Therefore, either the spread of effect from one hit commonly exceeds the limit of 15 bands conceived by DEMEREC and FANO, or else an excessive spread of effect is characteristic of heterochromatin. In future experiments it would be desirable to determine the length of the heterochromatic loss in the case of mutants induced in w^{m4} and rst^3 as well as to use other doses than 5000 r in order to distinguish between one- and two-hit effects.

The failure to produce any male-viable split mutations in the present experiment may seem surprising. Not one was detected among nearly 53,000 F_1 females heterozygous for *spl* while 174 Notch mutants were being found. This significant failure of X-rays to induce *spl* mutants suggests two things: a) split mutants arise only from true non-lethal gene changes affecting the locus whose loss gives rise to Notch, and b) X-rays are incapable of inducing true gene mutations at the spl^+ locus. Split, then, may be considered as an allele of Notch in the sense that every Notch is a deficiency of the spl^+ locus.

DEMEREC (1934) failed to induce any male-viable facet mutants although 27 Notch mutants (deficiencies of *fa*) were found. Thus, the relations of spl^+ to Notch very likely are duplicated by fa^+. All tested *N* mutants uncover both *spl* and *fa*, and X-rays may well be capable of producing neither male-viable *spl* nor male-viable *fa* mutants. It is improbable that a unitary N^+ locus exists,

although use of the symbol is convenient. The wild-type condition is better indicated as *spl*$^+$ *fa*$^+$, as suggested by BAUER (1943).

Can it be true that X-rays produce no true gene mutations at any locus in *Drosophila* (see LEFEVRE 1950)? Many male-viable mutants are found following irradiation, such as the whites in the present experiment. These viable white mutants are rarely associated with cytological abnormality, whereas lethal whites are nearly always detectably deficient (see DEMEREC 1941, and descriptions in BRIDGES and BREHME 1944). There are few, if any, "no-band" lethal whites. It is the viable whites which correspond to the cytologically normal lethal Notches. Reasons have been presented for believing that these Notches, like the detectably deficient Notches, result from real, though invisibly small, loss. The X-ray-induced male-viable whites may well result from the same cause. PANSHIN (1938) has reported the survival of a homozygous white deficiency; deficiency of yellow, too, is not necessarily lethal, according to MULLER (1935). Two categories of gene loci may be visualized. Some loci, represented by w^+ and y^+, are dispensable and may survive as deficiencies if neighboring loci are not simultaneously deleted. Other loci, represented by *spl*$^+$, are essential and any loss, however small, is lethal. Viable mutants at loci such as *spl*$^+$ arise only by true gene mutation, but true gene mutation is not produced by X-radiation.

SUMMARY

Following irradiation of Canton-S +, Abruptex, white-mottled-4 and roughest-3 males with 5000 r, white and Notch mutations were detected in the F_1 females. Approximately 25,000 F_1 females were examined in each series. The incidence of both white and Notch mutants produced in *Ax* was very similar to that observed in Canton-S +. Thus, the extra band in *Ax* does not contain a duplicated N^+ locus, and the Abruptex phenotype must result from a position effect. About 1/7 of all Notches simultaneously involve the white locus.

Both white and Notch effects were greatly increased when the inverted X-chromosomes were irradiated. In w^{m4} and rst^3 the N^+ locus lies close to proximal heterochromatin. The frequency of Notches induced in w^{m4} was similar to that in rst^3; being more than three times greater than the Notch frequency in the non-inverted X-chromosomes. In w^{m4} the excess number of Notches results from deficiencies extending into the heterochromatin. Therefore, proximity to heterochromatin facilitates the production of deletions of euchromatic loci viable as heterozygotes. In normal X-chromosomes Notch deficiencies longer than 50 salivary bands are not detected because such long deficiencies are dominant lethals. However, when the bulk of deleted material is heterochromatin, much longer deficiencies can survive in heterozygotes. It is not clear whether these long deficiencies result from one-hit or two-hit events.

Male-viable split mutations could have been detected in the w^{m4} and rst^3 irradiations. Nearly 53,000 F_1 females heterozygous for *spl* were examined, and while 174 Notch mutations were found, not one male-viable split mutation

was observed. The failure of X-rays to induce *spl* alleles suggests that X-rays do not give rise to true gene mutations but only to destructive changes. All Notch mutants likely result from deletion, even though many show no cytological loss. A *spl* mutant, however, must result from true mutation of the spl^+ locus, loss of which produces Notch.

In Canton-S + and *Ax* the total number of white mutants (viable and lethal) closely approximated the total number of Notch mutants. It may be suspected that male-viable white mutants are equivalent to the " no-band " Notches, and those induced by X-rays really result from genetic destruction rather than from true mutation. The loss of some loci, such as w^+ and y^+, is not necessarily lethal if the deletion does not include adjacent loci. The loss of other loci, such as spl^+, is invariably lethal.

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